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(54) Title: METHOD FOR TREATING OR PREVENTING ISCHEMIA-REPERFUSION INJURY

(57) Abstract

There is disclosed a method for the manufacture of a pharmaceutical composition for use in treating ischemia-reperfusion injury comprising admixing a pharmaceutically acceptable carrier and an effective amount of Interleukin-10.

METHOD FOR TREATING OR PREVENTING ISCHEMIA-REPERFUSION INJURY

BACKGROUND OF THE INVENTION

Ischemia-reperfusion injury frequently occurs when the flow of blood to a region of the body is temporarily halted (ischemia) and then re-established (reperfusion). Ischemia-reperfusion injury can occur during certain surgical procedures, such as repair of aortic aneurysms and organ transplantation. Clinically ischemia-reperfusion injury may be manifested by such complications as pulmonary dysfunction, including adult respiratory distress syndrome, renal dysfunction, consumptive coagulopathies including thrombocytopenia, fibrin deposition into the microvasculature and disseminated intravascular coagulopathy, transient and permanent spinal cord injury, cardiac arrhythmias and acute ischemic events, hepatic dysfunction including acute hepatocellular damage and necrosis, gastrointestinal dysfunction including hemorrhage and/or infarction and multisystem organ dysfunction (MSOD) or acute systemic inflammatory response syndromes (SIRS). The injury may occur in the parts of th body to which the blood supply was interrupted, or it can occur in parts fully supplied with blood during the period of ischemia.

International Patent Publication No. WO 96/01318 relates to polypeptides other than interleukin -10 (IL-10) allegedly having one or more properties similar to those of IL-10. Among the very long list of diseases allegedly treatable with these non-IL-10 proteins are tissue damage as a result of "hypoxia/ischemia (infarction: reperfusion)", "ischemia", "reperfusion injury", and "reperfusion syndrome". However, there is no evidence in this publication that the non-IL-10 proteins would actually work for treating all of the dis ases in the long list.

followed by reperfusion. Procedur s which involve such ischemia-reperfusion include but are not limited to repair of abdominal aortic aneurysms, aortic femoral, popliteal or tibial bypass for claudication or limb threatening ischemia, repair of popliteal or femoral aneurysms, bypass, thrombectomy or embolectomy for acute limb ischemia, or vascular trauma. Administration of IL-10 may improve limb salvage and survival after significant torso or extremity ischemia.

The amount of IL-10 to be administered is preferably between 0.1 to 500 µg/kg of body weight, more preferably 1 to 50 µg/kg. The IL-10 may be of human or viral origin produced biologically from mammalian cellular sources or by recombinant DNA technology. Administration preferably takes place by intravenous, intramuscular or subcutaneous injection. The IL-10 is preferably administered from one to zero hours before the blood flow is reestablished.

In those surgical procedures in which temporary or sustained disruption of blood flow is anticipated to occur, as before surgical repair of thoracoabdominal or supraceliac aneursymal disease, or surgical procedures to the abdomen that will necessarily include the transient reduction in visceral blood flow, or for organ transplantation, the IL-10 is preferably given either as a single bolus injection one to zero hours before the ischemic event or as a continuous intravenous injection beginning one to zero hours before the ischemic event and extending during th perioperative period and continuing for at least eight hours after restoration of visceral blood flow.

For individuals in whom disrupted visceral blood flow has already occurred, as in those individuals with trauma or injury to the visceral organs or their blood supply, or in patients with systemic hypotension due to shock, the IL-10 would be preferably given either as a single bolus injection prior to or simultaneously with restoration of normal visceral blood flow or as a continuous intravenous injection prior to or simultaneously with restoration of normal visceral blood flow and extending for at least eight hours after restoration of visceral blood flow.

For individuals in whom disrupted skeletal blood flow has already occurr d, as in those individuals with acute lower extremity ischemia due to embolic or thrombotic occlusion of peripheral blood vessels or acute ischemia due to vascular trauma, th IL-10 would be preferably given either as a single bolus injection prior to or simultan ously with restoration of normal blood flow or as a continuous intravenous injection prior to or simultaneously with restoration of

DETAILED DESCRIPTION OF THE INVENTION

All references cited herein are hereby incorporated in their entirety by reference.

As used herein, "interleukin-10" or "IL-10" is defined as a protein which (a) has an amino acid sequence of mature IL-10 (*e.g.*, lacking a secretory leader sequence) as disclosed in U.S. Patent No. 5,231,012 and (b) has biological activity that is common to native IL-10. Also included are muteins and other analogs, including the Epstein-Barr Virus protein BCRF1 (viral IL-10), which retain the biological activity of IL-10.

IL-10 suitable for use in the invention can be obtained from culture medium conditioned by activated cells secreting the protein, and purified by standard methods. Additionally, the IL-10, or active fragments thereof, can be chemically synthesized using standard techniques known in the art. See Merrifield, Science *233*:341 (1986) and Atherton *et al.*, *Solid Phase Peptide Synthesis: A Practical Approach*, 1989, I.R.L. Press, Oxford. See also U.S. Patent No. 5,231,012.

Preferably, the protein or polypeptide is obtained by recombinant techniques using isolated nucleic acid encoding the IL-10 polypeptide. General methods of molecular biology are described, *e.g.*, by Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor, New York, 2d ed., 1989, and by Ausubel *et al.*, (eds.) *Current Protocols in Molecular Biology*, Green/Woley, New York (1987 and periodic supplements). The appropriate sequences can be obtained using standard techniques from either genomic or cDNA libraries. Polymerase chain reaction (PCR) techniques can be used. See, *e.g.*, *PCR Protocols: A Guide to Methods and Applications*, 1990, Innis *et al.*, (Ed.), Academic Press, New York, New York.

Libraries are constructed from nucleic acid extracted from appropriate cells. See, *e.g.*, U.S. Patent No. 5,231,012, which discloses recombinant methods for making IL-10. Useful gene sequences can be found, *e.g.*, in various sequence databases, *e.g.*, GenBank and BMPL or nucleic acid and PIR and Swiss-Prot for protein, c/o Intelligenetics, Mountain View, California, or the Genetics Computer Group, University of Wisconsin Biotechnology Center, Madison, Wisconsin.

Clones comprising sequences that encode human IL-10 hav been deposited with the American Typ Culture Collection (ATCC), Rockville, Maryland, under Accession Nos. 68191 and 68192. Identification of other clones harboring

solubilizing the inclusion bodies with chaotropic agent and reducing agent so that the peptide assumes a biologically active conformation. For specifics of these procedures, see, *e.g.* Winkler *et al.*, Biochemistry *25*:4041 (1986), Winkler *et al.*, Bio/Technology *3*:9923 (1985); Koths *et al.*, and U.S. Patent No. 4,569,790.

The nucleotide sequences used to transfect the host cells can be modified using standard techniques to make IL-10 or fragments thereof with a variety of desired properties. Such modified IL-10 can vary from the naturally-occurring sequences at the primary structure level, *e.g.*, by amino acid, insertions, substitutions, deletions and fusions. These modifications can be used in a number of combinations to produce the final modified protein chain.

The amino acid sequence variants can be prepared with various objectiv s in mind, including increasing serum half-life, facilitating purification or preparation, improving therapeutic efficacy, and lessening the severity or occurrence of side effects during therapeutic use. The amino acid sequence variants are usually predetermined variants not found in nature, although others may be post-translational variants. Such variants can be used in this invention as long as they retain the biological activity of IL-10.

Modifications of the sequences encoding the polypeptides may be readily accomplished by a variety of techniques, such as site-directed mutagenesis (Gillman *et al.*, Gene *8*:81 (1987)). Most modifications are evaluated by routine screening in a suitable assay for the desired characteristics. For instance, U.S. Patent No. 5,231,012 describes a number of *in vitro* assays suitable for measuring IL-10 activity.

Preferably, human IL-10 is used for the treatment of humans, although viral IL-10 could possibly be used. Most preferably, the IL-10 used is recombinant human IL-10. The preparation of human IL-10 has been described in U.S. Patent No. 5,231,012. The cloning and expression of viral IL-10 (BCRF1 protein) from Epstein-Barr virus has been disclosed by Moore *et al.*, Science *248*:1230 (1990).

For examples of procedures and assays to determine IL-10 activity, see United States Patent No. 5,231,012. This patent also provides proteins having IL-10 activity and production of such proteins including recombinant and synthetic techniques.

To prepare pharmaceutical compositions of IL-10 for practice of this invention, th IL-10 is admixed with a pharmaceutically acceptable carrier or

following visceral ischemia and reperfusion in humans by measuring proinflammatory cytokine levels in patients undergoing thoracoabdominal or infrarenal aortic aneurysm repair, and comparing these results to the incidence of postoperative organ dysfunction.

Sixteen human patients undergoing elective repair of a thoracoabdominal aortic aneurysm and 9 patients undergoing elective infrarenal aortic aneurysm repair agreed to arterial blood sampling for proinflammatory cytokine measurements. Each thoracoabdominal aortic aneurysm was repaired through a left flank incision using a retroperitoneal approach. The diaphragm was divided circumferentially, allowing exposure of the descending thoracic aorta. Prior to cross-clamping, each patient was given mannitol (0.5 gm/kg) and solumedrol (15 mg/kg). Depending upon the location of the aneurysm, the visceral arteries were sewn onto the graft as a Carrel patch or as part of the proximal anastomosis with an extensive posterior taper to the graft. Once the repair was completed, coagulation products (platelets and fresh frozen plasma) were infused as needed. Preoperatively, a catheter was placed in the lumbar spinal column and cerebrospinal fluid drained to maintain intrathecal pressure at 5-10 cm water. Infrarenal abdominal aortic aneurysms were repaired transperitoneally using standard surgical techniques and the aorta was reconstructed using either a straight tube graft to the aortic bifurcation or a bifurcated graft to the internal/external iliac artery bifurcation.

In both groups of patients, arterial blood samples (7 ml) were obtained following induction of anesthesia, just prior to aortic cross-clamp placement, just prior to clamp release, and at timed intervals (1, 2, 4, 6 to 8, 24 hrs and daily for 7 days) after reperfusion. Clinical and laboratory data were collected prospectively from all patients to determine preoperative risk factors and postoperative organ dysfunction patterns. Data collected included operative parameters (total operative time, aortic cross-clamp time, estimated blood loss, intraoperative complications), postoperative course (complications, organ dysfunction) and causes of death. Laboratory values were analyzed during the initial 7 postoperative days to focus on the injury associated with tissue ischemia-reperfusion after thoracoabdominal and infrarenal aortic aneurysm repair.

Postoperative pulmonary dysfunction was defined as the need for positive-pressure mechanical ventilatory assistance for greater than 7 days while postoperative hepatic dysfunction was defined as peak lactate dehydrogenase

and placement of a temporary tracheostomy was eventually required in 4 patients. Renal dysfunction developed in 6 patients and hemodialysis was necessary in 2 of them. Hepatic dysfunction, thrombocytopenia, and leukopenia developed after thoracoabdominal aortic aneurysm repair in 5, 6, and 2 patients, respectively, and lower extremity dysfunction due to spinal cord injury occurred in 2 patients. In contrast, there were no operative deaths after infrarenal aortic aneurysm repair (Table 1). Pulmonary dysfunction occurred in only 1 patient and there was no evidence of renal, hepatic, hematopoietic or lower extremity dysfunction in any patient.

The peak plasma cytokine responses in both groups of patients are reported in Table 2.

TABLE 2

Peak proinflammatory cytokine concentrations following thoracoabdominal or infrarenal aortic aneurysm repair.

Plasma samples were obtained 0, 1, 2, 4, 6-8, 24, 48, 72 hours and daily for up to seven days following thoracoabdominal or infrarenal aortic aneurysm repair. Peak concentrations are reported here. Levels of all proinflammatory cytokines were significantly higher in patients following thoracoabdominal than infrarenal aortic aneurysm repair ($p<0.05$).

	Thoracoabdominal Aortic Aneurysm (n=16)	Infrarenal Aortic Aneurysm (n=9)
TNF-α pgs/ml	161±58	10±10
IL-1b pgs/ml	133±59	24±10
IL-6, pgs/ml	1,280±664	181±108
IL-8, pgs/ml	410±139	137±77
p55, change from baseline in pgs/ml	751±668	204±218
p75, change from baseline in pgs/ml	5,201±1,983	383±171
C3a, µg/ml	111±21	30±7

all values are significantly different between the two groups, by two-way ANOVA, $p<0.05$

Plasma TNF-α IL-1, IL-6 and IL-8 concentrations were undetectable prior to surgery. Following surgical repair of thoracoabdominal aortic aneurysms, a monophasic TNF-α response was detected in 11 of 16 patients (69%) (Figures 1(a), 1(b) and 1(c)). TNF-α levels peaked 4 hours after reperfusion and then gradually decreased toward baseline over the next 24 hours. IL-6 and IL-8 levels also increased in a monophasic pattern with peak levels again occurring 4 hours

In addition, patients who developed MSOD after thoracoabdominal aortic aneurysm repair had higher circulating levels of all assayed cytokine and soluble TNF-α receptors (p55 and p75) as compared to patients without MSOD (Table 4); however, only TNF-α and p55 receptor levels were statistically different ($p<0.05$) while there was a trend toward higher levels of IL-1, IL-6, IL-8 and p75 receptors in patients who developed MSOD as compared to patients without MSOD (Table 4).

TABLE 4

Plasma proinflammatory cytokine concentrations in patients with and without evidence of multisystem organ dysfunction (MSOD).

Peak plasma concentrations of TNF, IL-6, p55 and p75 were significantly higher in patients following thoracoabdominal aortic aneurysm repair with MSOD than in patients either following thoracoabdominal aortic aneurysm repair without MSOD or in patients following infrarenal aortic aneurysm repair.

	Thoracoabdominal Aortic Repair with MSOD	Thoracoabdominal Aortic Repair w/o MSOD	Infrarenal Aortic Repair
cross-clamp time	56±5 mins	33±4 mins	nr
TNFα	414±59*	86±55	10±10
IL-1ß	173±112	102±62	24±10
IL-6	4,907±1887*	344±66	181±108
IL-8	601±259	376±107	137±77
p55	+3,515±711*	+452±415	+204±218
p75	+9,469±1940*	+4,136±1,884	+382±171

values for p55 and p75 are changes from baseline. All values are in pgs/ml.
* $p<0.05$ versus no MSOD by 2-way ANOVA

nr = not reported

The results presented here demonstrate that surgical repair of thoracoabdominal aortic aneurysms which causes visceral ischemia-reperfusion injury results in a systemic proinflammatory cytokine response characterized by the appearance of TNF-α, IL-1, IL-6 and IL-8 in the blood as early as 1 to 4 hours after release of the cross-clamp. Additionally, the presence and magnitude of this proinflammatory cytokine response is associated with the incidence of postoperative organ dysfunction after thoracoabdominal aortic aneurysm repair. Ischemia and subsequent reperfusion injury of the viscera appear to be critical for the induction of this systemic proinflammatory cytokine respons , because the magnitude of the proinflammatory cytokine response is 3 to 15-fold less in patients undergoing repair of the infrar nal aorta where visceral ichemia/reperfusion do s

IL-8, pgs/ml	410±139	458±402
IL-10, pgs/ml	60±29	806±260*
p55, baseline pgs/ml	+741±298	+751±668
p75, baseline pgs/ml	+641±406	+5201±1983*

* $p<0.05$

Additionally, the incidence of pulmonary dysfunction, renal dysfunction, thrombocytopenia, multisystem organ dysfunction, and mortality were reduced in patients undergoing LAFBP, although the numbers were too small to show any statistical difference.

These findings suggest that acute visceral ischemia-reperfusion injury secondary to thoracoabdominal aortic aneurysm repair is associated with a high rate of morbidity and multisystem organ dysfunction that is not seen with similar surgical procedures that do not cause visceral ischemia. Furthermore, techniques aimed at reducing the duration of ischemia during aortic cross-clamping (left atrial-femoral bypass) appear to reduce the magnitude of the TNF-α and IL-1 responses.

EXAMPLE 2

Experiments in mice have been conducted that demonstrate that pretreatment with recombinant human IL-10 can reduce distant organ injury in a clinically relevant model of acute visceral ischemia-reperfusion injury. The initial goal of these studies was to develop a clinically relevant model of acute ischemia-reperfusion injury that demonstrated evidence of organ injury that was dependent upon an endogenous proinflammatory cytokine response that could be inhibited by either a TNF-α receptor construct or a monoclonal antibody against the IL-1 type I (p80) receptor (35F5, Hoffmann-LaRoche, Nutley, NJ).

Thirty mice (C57BL/6, approx. 20 gm) were anesthetized with pentobarbital. In 16 of these animals, the supraceliac aorta was cross-clamped for 30 minutes. Six animals had th ir infrarenal aorta cross-clamped for 30 minutes, while another 8 animals received only anesthesia, incision and bow l mobilization without aortic cross-clamping. Two hours prior to supraceliac aortic cross-clamping, 8 of the 16 animals were pretreated with the intraperitoneal injection of 10 mg/kg BW of TNF-

Plasma IL-10 levels were measured by ELISA at 1, 2, 4 and 8 hrs after reperfusion, and lung neutrophil infiltration was determined by MPO assay at 2 hrs, as previous studies had revealed that maximal neutrophil infiltration occurred in the lung at 2 hrs. Thirty-six of the mice undergoing visceral ischemia-reperfusion were pretreated with 0.2μg (n=7), 2μg (n=13), 5μg (n=6), or 20μg (n=10) of recombinant human IL-10.

Mean plasma IL-10 concentrations peaked at 9,120 pg/ml 2 hours following 25-30 minutes of supraceliac aortic cross-clamping (Figure 5). Visceral ischemia-reperfusion injury also resulted in an 6-fold increase in lung neutrophil infiltration ($p<0.05$) (Figure 6). When mice were pretreated with exogenous IL-10, neutrophil infiltration was significantly reduced ($p<0.05$ for all doses). Maximal improvements in pulmonary neutrophil infiltration were attained with 5 μg/mouse (250 μg/kg BW) of IL-10.

Visceral ischemia-reperfusion injury associated with supraceliac aortic cross-clamping promotes the release of IL-10, while exogenous IL-10 administration prior to aortic cross-clamping limits pulmonary injury in this model of acute visceral ischemia-reperfusion injury. Thus, exogenous IL-10 may offer a novel therapeutic approach to decrease complications associated with thoracoabdominal aortic aneurysm repair and other ischemia-reperfusion injuries.

Hypothetical Example 3 illustrates a preferred application of the invention contemplated for treating humans.

EXAMPLE 3

A 58 year-old white male presents to the emergency room of a local University hospital complaining of several months of intermittent sharp epigastric and periumbilical abdominal pain, with no other significant symptoms. The pati nt has no history of any significant medical problems other than a history of atherosclerotic disease. On physical exam, the patient is found to have a nontender, pulsatile mid-abdominal mass, with an audible bruit. Laboratory examination including hematology, biochemistries, liver function tests, urinalysis and amylase are all within normal limits. Flat and upright abdominal x-rays, as well as chest x-rays, are unremarkable. An abdominal CT scan with cuts through the lower chest reveals an aortic aneurysm extending from the level of the diaphragmatic hiatus to the aortic bifurcation, 6.5 cm in largest diameter.

EXAMPLE 4

The following experiments in rats demonstrate that pretreatment with exogenous human IL-10 may decrease lung and soleus muscle injury in a clinically relevant model of hindlimb ischemia-reperfusion injury.

Twenty eight male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA., approx. 350 gm) were anesthetized with pentobarbital intraperitoneally (40 mg/kg, Abbott Laboratories, Chicago, IL.). In twenty of the rats, bilateral hindlimb ischemia was produced by placement of a rubber band tourniquet across the upper thigh of both lower extremities. The cessation of arterial blood flow was confirmed by the absence of a Doppler signal in the superficial femoral artery. The remaining eight rats received anesthesia alone.

Half of the animals in each group (10 in the ischemic group and 4 of the non-ischemic controls) were pretreated with 10 µg of recombinant IL-10. After the induction of anesthesia, a catheter was placed into the right atrium through th external jugular vein for blood sampling and infusion of normal saline (1 cc/hr). Recombinant human IL-10 (rhIL-10, 10 µg approx. 30 µg/kg BW IV) or a comparable volume of normal saline was administered twenty minutes prior to th onset of ischemia or at comparable times for the non-ischemic controls.

After 4 hours of ischemia, the tourniquets were removed and the extremity was reperfused. The restoration of arterial blood flow was confirmed by the presence of a Doppler signal in the superficial femoral artery. Blood (0.5 cc) was sampled at the time of central venous line placement, at reperfusion, 30 minutes after reperfusion, 60 minutes after reperfusion, and hourly thereafter. Blood was sampled at comparable time periods in the non-ischemic controls.

The animals were euthanized (pentobarbitol 100 mg/kg BW IV) after 4 hours of reperfusion or at comparable times for the non-ischemic controls. The soleus muscle from one hindlimb and one lung were analyzed for assessment of neutrophil infiltration. Soleus muscle and pulmonary neutrophil sequestration were quantified by the tissue myeloperoxidase (MPO) levels (Warr n *et al.*, 1989, J.Clin.Invest. 84:1873).

The remaining soleus muscle and lung tissue were analyzed to quantify the capillary and/or cellular injury. Skeletal muscle and lung capillary endothelial cell

Table 6
Skeletal Muscle Injury

	Muscle Capillary Permeability Index	Skeletal Muscle Injury Index
I/R	1.51 ± 1.46	0.94 ± 0.83
I/R + IL-10	0.39 ± 0.38*	0.36 ± 0.51
SHAM	0.03 ± 0.01*	0.05 ± 0.02
SHAM + IL-10	0.19 ± 0.23*	0.26 ± 0.30

* significantly different from I/R (ANOVA, Duncan's multiple range test; p<.05)

Lung Injury

The results are shown in Table 7. The hindlimb ischemia-reperfusion also resulted in significant pulmonary vascular injury as determined by the leakage of I^{125} albumin into the lungs. Both the mean pulmonary capillary permeability ind x and the mean pulmonary neutrophil infiltration in the animals subjected to hindlimb ischemia-reperfusion were significantly greater than the non-ischemic controls. Pretreatment with human recombinant IL-10 significantly reduced the lung capillary injury after hindlimb ischemia-reperfusion and the PCPI values in the pretreated animals were not different from the non-ischemic controls. In contrast, pretreatment with human recombinant IL-10 resulted in a significant increase in the lung myeloperoxidase content after hindlimb ischemia-reperfusion. Although a ready explanation for this latter finding is not immediately forthcoming and it is in no way essential to this invention, it may well have been that IL-10 prevented the activation and degranulation of neutrophils in the lung. In this model, IL-10 may not have prevented the recruitment of neutrophils into the lung, but prevented the degranulation of their toxic contents, thus explaining both the higher MPO levels and reduced endothelial injury. Treatment of the non-ischemic controls with human recombinant IL-10 also increased the pulmonary neutrophil infiltration, although this difference was not significant.

CLAIMS

1. The use of IL-10 for the manufacture of a medicament for treating or preventing ischemia-reperfusion injury.

2. A method for the manufacture of a pharmaceutical composition for treating or preventing ischemia-reperfusion injury, comprising admixing a pharmaceutically acceptable carrier and an effective amount of IL-10.

3. The use or method of claim 1 or 2 wherein the ischemia-reperfusion injury is caused by a major organ transplant or repair of an aneurysm.

4. The use or method of claim 1 or 2 wherein the ischemia-reperfusion injury is caused by surgical repair of a thoracic aortic aneurysm, a suprarenal aortic aneurysm, liver, kidney, small intestine, or pancreas transplant, hepatic and biliary surgical resections, total or partial pancreatectomy, total and partial gastrectomy, esophagectomy, colorectal surgery, vascular surgery for mesenteric vascular disease, abdominal insufflation during laparoscopic surgical procedures, blunt or penetrating trauma to the abdomen including gun shot wounds, stab wounds or penetrating wounds or blunt abdominal trauma secondary to deacceleration injury and/or motor vehicle accidents, hemorrhagic shock due to blood loss, cardiogenic shock to myocardial infarction or cardiac failure, neurogenic shock or anaphylaxis.

5. The use or method of claim 1 or 2 wherein the ischemia-reperfusion injury is caused by surgical repair of an abdominal aortic aneurysm, aortic femoral, popliteal or tibeal bypass for claudication or limb threatening ischemia, repair of popliteal or femoral aneurysms, bypass, thrombectomy or embolectomy for acute limb ischemia, or vascular trauma.

6. The method or use of any of claims 1-5 wherein the IL- 10 is human or viral IL-10.

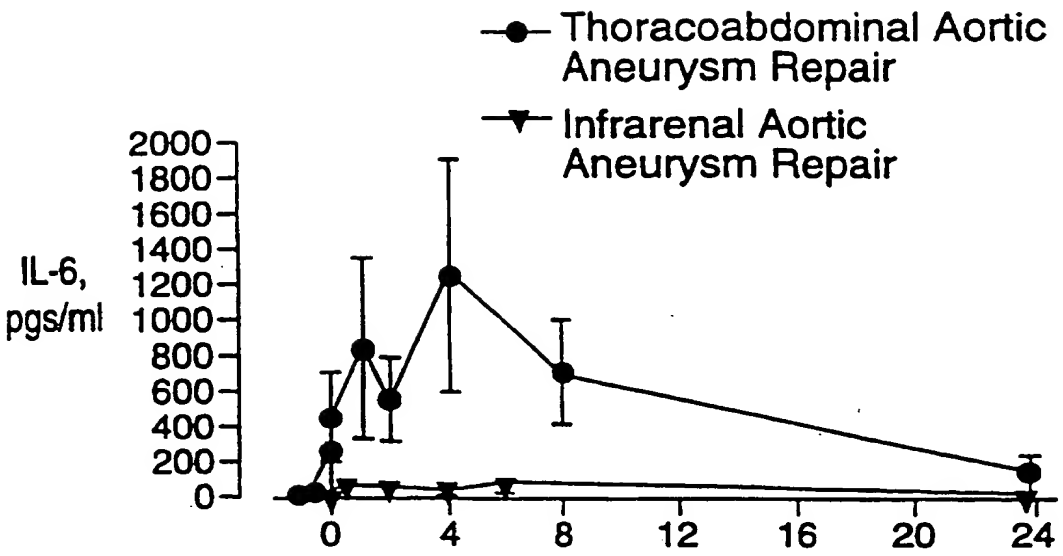

FIG. 2(a)

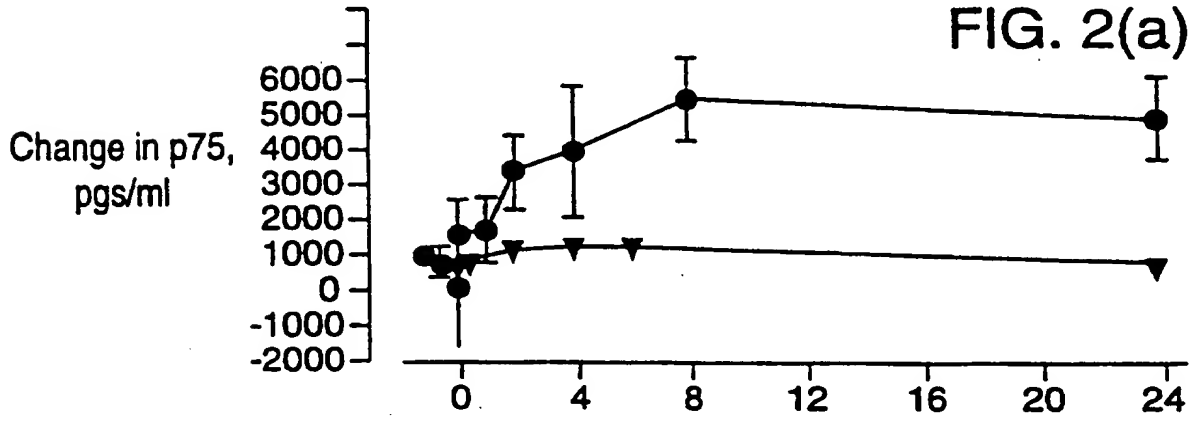

FIG. 2(b)

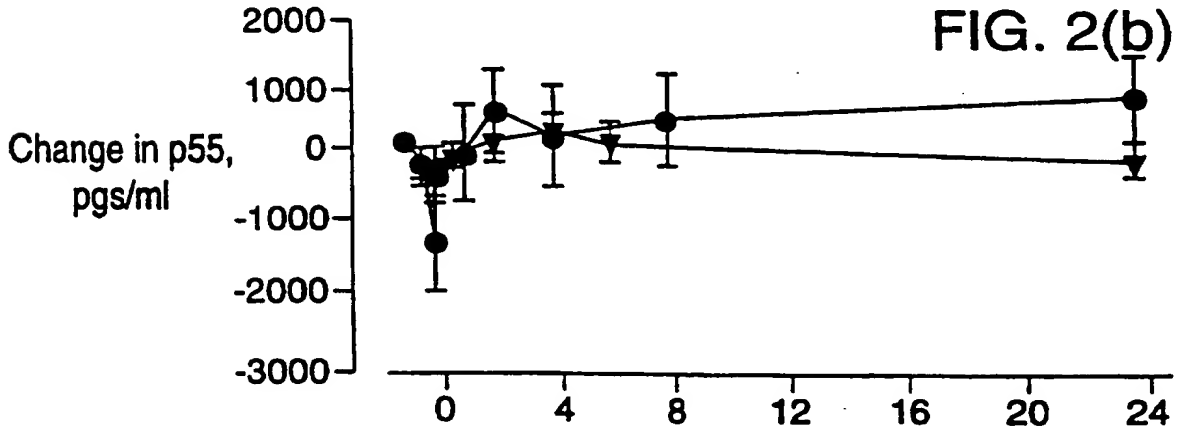

FIG. 2(c)

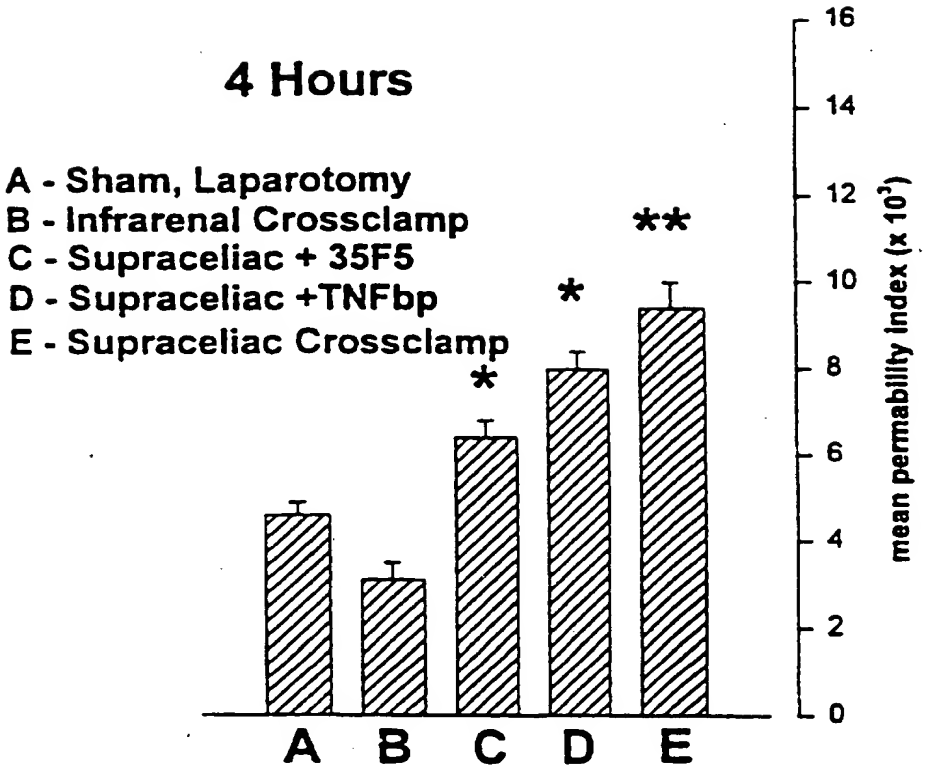

Fig. 4

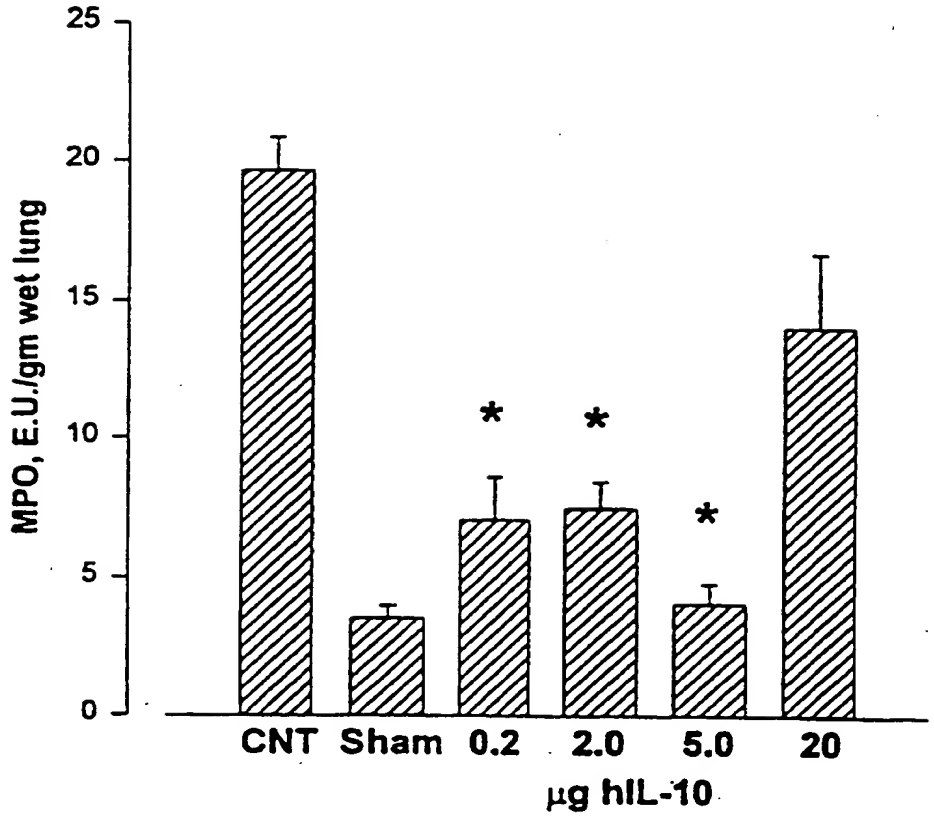

Fig. 6

INTERNATIONAL SEARCH REPORT

Inte ›nal Application No
PCT/US 97/06809

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 01318 A (NYCOMED DAK A/S) 18 January 1996 cited in the application see the whole document	1-6
P,X	6TH INTERNATIONAL TUMOR NECROSIS FACTOR CONGRESS, RHODES, GREECE, MAY 8-12, 1996. EUROPEAN CYTOKINE NETWORK 7 (2). 1996. 294, XP002035706 HESS P ET AL: "Exogenous IL - 10 suppresses tumor necrosis factor (TNF) dependent pulmonary neutrophil infiltration in a mouse model of visceral ischemia - reperfusion injury." see the whole document	1-6
P,X	J. THORAC. CARDIOVASC. SURG. (1996), 112(5), 1301-1306, 1996, XP002035707 EPPINGER, MICHAEL J. ET AL: "Regulatory effects of interleukin - 10 on lung ischemia - reperfusion injury" see the whole document	1-6

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